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***Mycobacterium tuberculosis* Beijing Type Mutation Frequency**

To the Editor: A striking finding in the study by de Steenwinkel et al. (1) is the high frequency of mutation to rifampin resistance by 2 *Mycobacterium tuberculosis* Beijing strains, which might play a role in the association between the Beijing strains and multidrug-resistant tuberculosis. Earlier reported frequency of mutation to rifampin resistance by *M. tuberculosis* has been 10^{-8} CFU (2,3), including the Beijing genotype (3,4). Of note, the Beijing 2002–1585 strain, for which frequency of mutation to rifampin resistance is 10^{-3} CFU (1 mutant/1,000 CFU), showed a moderate frequency of 10^{-8} CFU in another study (4). We think that a mutation frequency increase of $100,000\times$ is remarkably high. In contrast, rifampin-resistant mutants of the Beijing 1585 strain did not emerge in low-density cultures (5×10^5 CFU/mL) used for time-kill kinetics experiments, al-

though frequency of mutation to rifampin resistance was determined to be 10^{-3} CFU.

Mutation frequency is determined by fluctuation assays. To exclude preexisting mutants, which would bias the mutation frequency by so-called jackpots, a series of low-inoculum cultures is typically used (5). However, for unknown reasons, de Steenwinkel et al. used only 1 high-density culture of 10^{10} CFU of each strain to determine mutation frequency. This strategy is not recommended because mutations can occur early or late, resulting in substantial mutation frequency fluctuation between test episodes. A strain with known mutation rates should preferably be included to rule out possible technical errors.

We propose the following explanations for the remarkable results: 1) the rifampin concentration for selecting mutants might have been too low, enabling growth of some colonies of drug-susceptible bacteria; 2) rifampin mutants arose early or preexisted in the cultivation of Beijing strains 1585 and 1607, producing jackpots; or 3) the 2 Beijing isolates might contain rifampin-resistant subpopulations (heteroresistance). The capacity of the Beijing strain to develop and, especially, transmit multidrug-resistant tuberculosis remains to be further analyzed.

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In Response: We explain the differing frequencies of mutation to rifampin resistance mentioned by Werngren (1). First, the strains of *Mycobacterium tuberculosis* that we tested differed from those previously tested (2). Second, we used different rifampin concentrations in subculture plates. For Beijing strain 2002–1585, Bergval et al. (3) found a mutation frequency of $4\text{--}24 \times 10^{-8}$ at a subculture concentration of 8 mg/L, whereas we found a mutation frequency of $3\text{--}4 \times 10^{-3}$ at a subculture concentration of 1 mg/L and a lower mutation frequency at 2 mg/L. Thus, the concentration of drugs in subculture plates is crucial to mutation frequency assays. Absent a subculture concentration standard, we applied rifampin at 1 mg/L (4) because bacteria growing at this concentration are considered resistant to rifampin. Our mutation frequency and time-kill kinetics assay results are not contradictory